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METHOD FOR DECREASING DEPRESSION BYINHIBITING THE ACTIVITY OFN-TYPE CALCIUM CHANNEL

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FIELD OF THE INVENTION

The present invention relates to a method for decreasing depression, more particularly to a method for decreasing depression by inhibiting the activity of N-type calcium channel.

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BACKGROUND

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There are many different kinds of voltage-dependent calcium channels (VDCC) that play important roles in the control of neurotransmitter release, membrane excitability, and gene expression (Catterall, *Cell Calcium*, 1998, 24, 307-323). These channels have multi-subunit structures consisting of  $\alpha 1$ ,  $\alpha 2$ ,  $\beta$  and  $\gamma$  subunits (Dunlap et al., *Trends Neurosci.*, 1995, 18, 89-98; De Waard et al., *Ion Channels*, 1996, 4, 41-87; Hofmann et al., *Rev. Physiol. Biochem. Pharmacol.*, 1999, 139, 33-87). VDCCs are classified into various types including L-type, N-type, P-type, Q-type, R-type and T-type according to their

electrophysiological or pharmaceutical characteristics (Tsien et al., *Soc. Gen. Physiol. Ser.*, 1987, 41, 167-187; Randall and Tsien, *J. Neurosci.*, 1995, 15, 2995-3012). In general, L-type, N-type, P/Q-type and R-type channels are open with a high voltage, and T-type channel is open with a low voltage. Among voltage-dependent calcium channel subunits, alpha-1 subunit is essential for the channel operation and for the determination of the characteristics of the channel. As of today, 6 different genes (alpha 1A - alpha 1F), encoding in variety of sub-groups of alpha 1 subunit, have been disclosed (Chin., *Exp. Mol. Med.*, 1998, 30, 123-130; Ertel et al., *Neuron*, 2000, 25, 533-535; Hofmann et al., *Rev. Physiol. Pharmacol.*, 1999, 139, 33-87).

*In situ* hybridization in rat brains has shown that alpha 1B subunit is widely distributed in various regions of the central nervous system, especially in the dorsal raphe and locus coeruleus regions (TanaKa et al., *Brain Res. Mol. Brain Res.*, 1995, 30, 1-16). At the subcellular level, alpha 1B subunit is highly localized at the presynaptic nerve terminals of most neurons, consistent with its physiological role in regulating neurotransmitter release (Miller, *Science*, 1987, 235, 46-52; Hirning et al., *Science*, 1988, 239,

57-61; Westenbroek et al., *J. Neurosci.*, 1995, 15, 6403-18). In addition, the subunit has also been known to regulate neurotransmission in synapse by being involved in the inhibiting activity of serotonin and norepinephrine in presynapse or in postsynapse (Okada, et al., *J. Neurosci.*, 2001, 21, 628-640; Rittenhous A.R., *J. Neurobiol.*, 1999, 40, 137-148; Sun, Q.Q., et al., *J. Physiol.*, 1998, 510, 103-20).

Recently, results of genetic studies have been reported, which identify the accurate role of N-type calcium channel in handling of nociceptor information in vivo by using a mutant mouse deficient in alpha 1B subunit of N-type calcium channel by gene targeting method (Kim et al., *Mol. Cellular Neuroscience*, 2001, 18, 235-245; Saegusa, H. et al, *EMBO J.*, 2001, 15, 2349-56; Hatakeyama, S. et al, *Neuroreport*, 2001, 8, 2423-7). According to those studies, the alpha 1B mutant mouse felt a pain much less than a normal mouse, especially for a mechanical pain, a pain by radiant heat and an inflammatory pain. Besides, the mutant mouse was insensitive to a pain accompanied by a harmless stimulation caused by nervous injury and a pain by fever, comparing to a normal mouse. Thus, N-type calcium channel seems to play an important role

in recognizing a pain. Another report stated that alpha 1B mutant mouse was confirmed by the elevated plus maze test to feel uneasiness less than a normal mouse (Saegusa, H. et al, *EMBO J.*, 2001, 15, 2349-56).

5 However, pharmacological or genetic studies concerning the effect of N-type calcium channel on emotional troubles such as depression, etc. have not been reported yet.

10 Thus, the present inventors performed in variety of animal behavior tests relating to depression by using a mutant mouse deficient in N-type calcium channel alpha 1B gene, in order to investigate the relation between depression, a  
15 representative emotional trouble, and N-type calcium channel. And the present inventors have completed this invention by confirming that the inhibition of the activity of N-type calcium channel resulted in the reduction of the symptoms of depression.

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#### SUMMARY OF THE INVENTION

It is an object of this invention to provide a method for decreasing depression by inhibiting the activity of N-type calcium channel.

25 It is also an object of this invention to

provide a method for screening an anti-depression agent by using an alpha 1B gene of N-type calcium channel or a protein thereof.

5 It is a further object of this invention to provide an anti-depression agent containing an inhibitor of N-type calcium channel as an effective ingredient.

#### DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

10 In order to achieve the above object of the present invention, the present invention provides a method for decreasing depression by inhibiting the activity of N-type calcium channel.

15 The present invention also provides a method for screening an anti-depression drug by using an alpha 1B gene of N-type calcium channel or a protein thereof.

20 The present invention further provides an anti-depression drug containing an inhibitor of N-type calcium channel as an effective ingredient.

Hereinafter, the present invention is described in detail.

1. The present invention provides a method for  
25 decreasing depression by inhibiting the activity of

N-type calcium channel.

The above method for inhibiting the activity of N-type calcium channel might be one of these; administering a subject working specifically toward  
5 N-type calcium channel to inhibit its activity, injecting an antibody specifically binding to N-type calcium channel, inhibiting the transcription of a gene coding N-type calcium channel or inhibiting the translation of the transcribed N-type calcium channel  
10 gene, but not always limited thereto. Other methods that can inhibit the activity of N-type calcium channel are available. In the preferred embodiment of the present invention; the present inventors investigated the relation between depression and the  
15 inhibition of the activity of N-type calcium channel by using a mouse in which N-type calcium channel was not expressed because an alpha 1B gene coding N-type calcium channel was targeted (see FIG. 3 and FIG. 4).

20 In order to confirm whether N-type calcium channel was related to depression, a representative emotional trouble, the present inventors performed a forced swimming test and a tail suspension test, which are both representative animal behavior tests  
25 relating to depression, by using alpha 1B-knockout mice (Korea patent application #2002-2343). First,

as for the forced swimming test, both a mutant mouse and a normal mouse were forced to swim in a beaker for 15 minutes. As a result, while a normal mouse began to show the immobile floating position in 5 minutes, an alpha 1B mutant (knockout) mouse was swimming for 15 minutes all along, suggesting that the immobile floating position time of the mutant mouse was shorter. Thus, it was confirmed that depression in the mutant mouse was remarkably decreased, comparing to that in the normal mouse (see FIG. 3). Second, as for the tail suspension test, the result was similar to that of the forced swimming test, that is, the mutant mouse showed shorter immobilization time than the normal mouse (see FIG. 4). As explained hereinbefore, an alpha 1B-knockout mouse, in which a gene coding N-type calcium channel was targeted, showed an anti-depression response in the two depression related behavior tests above.

It has recently been disclosed that the abnormality of regulation of monoamines such as serotonin and norepinephrine, and substance P, an peptide, is related to depression pathologically and physiologically, so that a substance regulating such neurotransmitters is a prominent candidate for a depression treatment agent (Okada, et al., J.

Neurosci., 2001, 21, 628-640; Rittenhous A.R., J. Neurobiol., 1999, 40, 137-148; Sun, Q.Q., et al., J. Physiol., 1998, 510, 103-20). The above alpha 1B-knockout mouse, in which the activity of N-type calcium channel was inhibited, showed the equal behavior response to a normal mouse treated with an anti-depression agent such as selective serotonin reuptake inhibitors (SSRI), tricyclics, monoamine oxidase inhibitors, substance P or NK-1 receptor inhibitor. Serotonin 1A receptor mutant mouse showed much increased anxiety but decreased depression, comparing to a normal mouse. Norepinephrine transporter mutant mouse also showed decreased depression comparing to a normal mouse (Ramboz, S. et al., Proc. Natl. Acad. Sci. USA, 1998, 95, 14476-81; Xu, F. et al., Nature Neuroscience, 2000, 3, 465-471).

Other pharmacological or genetic researches concerning depression or anxiety informed us that depression or anxiety could be decreased by inhibiting substance P which was known to be secreted by N-type calcium channel related to a pain and NK-1 receptor thereof (Santarelli, L. et al., PNAS USA, 2001, 98, 1912-17). Nevertheless, the relation between depression and N-type calcium channel has not been clearly explained yet. Therefore, the present inventors have performed depression related animal

behavior tests using a mutant mouse and have clarified for the first time that depression can be decreased by inhibiting the activity of N-type calcium channel.

5           It was also reported from other studies on other calcium channels related to the regulation of an emotion that L-type calcium channel inhibitor 'nimodipine' alleviated ultra-rapid-cycling bipolar of bipolar diseases and brief recurrent depression,  
10       and a calcium channel alpha 2-delta inhibitors 'gabapentin' and 'lithium' could also be excellent candidates for the treatment agent of such emotional disorder (Post, R.M., et al., *Bipolar Disord.*, 2000, 2, 305-15; Goodnick, P.J., *Biopolar Disord.*, 2000, 2,  
15       165-73). However, as of today, no reports concerning in vivo function of a calcium channel related to depression have been made, except the result of the present invention. So, the present inventors have first confirmed the fact that the inhibition of the  
20       activity of N-type calcium channel is related to decreasing depression.

          In conclusion, the inhibition of the activity of N-type calcium channel by suppressing the  
25       expression of an alpha 1B protein results in the anti-depression response, suggesting that an N-type

calcium channel gene activity inhibitor or its product N-type calcium channel activity inhibitor might be used for the development of a depression treatment agent.

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11. The present invention provides a method for screening an anti-depression agent by using a N-type calcium channel alpha 1B gene or a protein thereof.

If ever found, an alpha 1B activity inhibitor  
10 can be effectively used as an anti-depression agent. Thus, it is possible to screen a substance inhibiting the activity of an alpha 1B by taking advantage of the general screening method for a substance inhibiting the activity of a protein. The general  
15 method for the screening of a substance inhibiting the activity of an alpha 1B is composed of the following steps:

1) Obtaining a transformant by transfecting host cells with a vector containing an alpha 1B  
20 structural gene and a reporter gene;

2) Culturing the above transformant along with a test sample for screening; and

3) Measuring the expression of the reporter gene.

25 As a reporter gene in the above, LacZ, GFP and luciferase could be used, but not always limited

thereto.

III. The present invention provides an anti-depression agent containing a N-type calcium channel  
5 inhibitor as an effective ingredient.

As a N-type calcium channel inhibitor, a compound acting specifically upon N-type calcium channel to inhibit its activity, an antibody combining specifically with N-type calcium channel, a  
10 substance inhibiting the transcription of a gene encoding N-type calcium channel or a substance inhibiting the translation of a transcribed N-type calcium channel gene can be selected, but the selection is not always limited thereto, and further  
15 any other substances that can inhibit the activity of N-type calcium channel can be used.

The suppressor of N-type calcium channel according to the present invention can be  
20 administered orally or parenterally and be used in general form of pharmaceutical formulation. The N-type calcium channel suppressor of the present invention can be prepared for oral or parenteral administration by mixing with generally used fillers, extenders, binders, wetting agents, disintegrating  
25 agents, diluents such as surfactants, or excipients.

Solid formulations for oral administration are tablets, pills, dusting powders and capsules. These solid formulations are prepared by mixing one or more suitable excipients such as starch, calcium carbonate, sucrose or lactose, gelatin, etc. Except for the simple excipients, lubricants, for example magnesium stearate, talc, etc, can be used. Liquid formulations for oral administrations are suspensions, solutions, emulsions and syrups, and the abovementioned formulations can contain various excipients such as wetting agents, sweeteners, aromatics and preservatives in addition to generally used simple diluents such as water and liquid paraffin. Formulations for parenteral administration are sterilized aqueous solutions, water-insoluble excipients, suspensions, emulsions, and suppositories. Water insoluble excipients and suspensions can contain, in addition to the active compound or compounds, propylene glycol, polyethylene glycol, vegetable oil like olive oil, injectable ester like ethylolate, etc. Suppositories can contain, in addition to the active compound or compounds, witepsol, macrogol, tween 61, cacao butter, laurin butter, glycerinated gelatin, etc.

The mentioned N-type calcium channel suppressor can be mixed with many pharmaceutically acceptable

carriers such as physiological saline or organic solvent, and can additionally include carbohydrates such as glucose, sucrose or dextran, antioxidants such as ascorbic acid or glutathion, chelating agents, low molecular proteins or other stabilizers to enhance stability or absorptiveness.

The effective dose of N-type calcium channel suppressor of the present invention can be administered in the form of bolus, by single dose having relatively short period of infusion or by multiple dose of fractionated treatment protocol for a long term. The decision of an effective dosage of the N-type calcium channel suppressor depends on the administration pathway, treatment times, age and other conditions of a patient, etc. Therefore, any expert who has knowledge on this field can decide the effective dosage of the N-type calcium channel suppressor of the present invention.

The preferable effective dosage of N-type calcium channel suppressor of the present invention is 0.014~0.14  $\mu\text{g}/\text{kg}$  for an injection and 0.14~1.4  $\mu\text{g}/\text{kg}$  for oral administration, and administration times are 1~6 per day (Richard D.P. and Judith A.P. Pain, 2000, 85, 291-296).

BRIEF DESCRIPTION OF THE DRAWINGS

The application of the preferred embodiments of the present invention is best understood with reference to the accompanying drawings, wherein:

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FIG. 1 is a schematic diagram showing the structures of wild-type alpha 1B locus, targeting vector and disrupted alpha 1B locus,

A: Wild-type alpha 1B gene locus,

10 B: Targeting vector in which IS3 region is replaced with GEP-Neo and tk gene is included,

C: Gene locus targeted with targeting vector,

□: Fragment used as a probe

15 FIG. 2A is an electrophoresis photograph showing the result of PCR of wild-type, heterozygous, and homozygous mutant mice,

Lane 1: Marker,

Lane 2: Wild-type(+/+),

20 Lane 3: Heterozygote(+/-),

Lane 4: Homozygote(-/-)

FIG. 2B is an electrophoresis photograph showing the result of Southern blot analysis of wild-type,  
25 heterozygous, and homozygous mutant mice,

Lane 1 and 2: Wild-type(+/+),  
Lane 3 - 5 : Heterozygote(+/-),  
Lane 6 and 7: Homozygote(-/-)

5        FIG. 2C is an electrophoresis photograph showing  
the result of Western blot analysis of brain tissues  
from wild-type and transgenic mice,

Lane 1: Cerebellum of wild-type mice(+/+),  
Lane 2: Cerebellum of transgenic mice(-/-),  
10       Lane 3: Cerebrum of wild-type mice(+/+),  
Lane 4: Cerebrum of transgenic mice(-/-)

FIG. 3 is a graph showing the result of forced  
swimming test performed with wild-type and alpha 1B-  
15       knockout mice,

FIG. 4 is a graph showing the result of tail  
suspension test performed with wild-type and alpha  
1B-knockout mice.

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#### EXAMPLES

Practical and presently preferred embodiments  
of the present invention are illustrative as shown in  
the following Examples.

25       However, it will be appreciated that those

skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

5 Example 1: Generation of alpha 1B-knockout mice

<1-1> Generation of targeting vector deficient in calcium ion channel alpha 1B gene

In order to generate knockout mice for the alpha 1B gene of calcium ion channel, the present  
10 inventors had performed gene targeting method. First, bacteriophage lamda FIX II library (Stratagene) wherein DNA fragments of 129/sv mouse genome were inserted randomly was screened with alpha 1B rat cDNA (AUG ~ 720 bp, obtained from Dr. Jin HM, NIH) as a  
15 probe to obtain mouse genomic DNA containing alpha 1B gene. Through the screening, bacteriophage clone having 18.4 kb genomic DNA containing the intervening segment (IS) 1-3 coding region of the alpha 1B locus was obtained, which was later used for the generation  
20 of a targeting vector after completing a restriction enzyme map using various restriction enzymes. pBluescript II KS(+/-) (Stratagene) was used as an empty vector. From the pEGFP-1 vector (Clontech), green fluorescent protein (GFP) gene containing an  
25 SV-40-polyA was cut and fused to the *Bam*HI-tagged

IS2 containing exon (amino acid 158). The PGK-neo gene originated from pLNT vector (obtained from Sugitau Noda) was attached to downstream of the GFP.

As a result, a targeting vector wherein some parts of IS3 were deleted and replaced with the GFP-NEO cassette for gene disruption and positive selection was constructed. For negative selection, the HSB-tk gene was attached at the 5' end of the 5'-homology region (FIG. 1).

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#### <1-2> Transfection into embryonic stem cells

J1 embryonic stem cell line was used for the transfection of targeting vector generated in the above Example <1-1>. Embryonic stem cell cultures and embryo manipulations were performed as described in the following reference (Kim et al., Nature, 1997, 389, 290-293). J1 embryonic stem cells (obtained from Dr. R. Jeanisch, MIT, USA) were maintained in DMEM (Gibco Co.) supplemented with 15% fetal bovine serum (Hyclone Co.), 1× penicillin-streptomycin (Gibco Co.), 1× non-essential amino acid (Gibco Co.) at 37°C for 2-3 days. Single cells were obtained by treating the cells with 1 mM EDTA solution containing 0.25% trypsin.

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Targeting vector generated in the above Example

<1-1> was transfected into the single cells by electroporation. Particularly, 25  $\mu$ g of targeting vector DNA was added into embryonic stem cells ( $2 \times 10^7$  cells/ml). After mixing, electroporation was performed with 270 V/500  $\mu$ F. The cells were cultured in ES medium containing 0.3 mg/ml of G418 and 2  $\mu$ M of gancyclovir for 5-7 days. Embryonic stem (ES) cell clones correctly targeted were selected by using homologous recombination method, and maintained. After confirming the defection in calcium ion channel alpha 1B gene, the embryonic stem cell clones were distributed into the ES medium again for further culture for 18-22 hours. Single cells were obtained by treating the cells with trypsin. Among them, just live cells were used for microinjection.

#### <1-3> Generation of chimera mice

In order to generate chimera mice having alpha 1B +/- genotype, embryonic stem cell clone selected in the above Example <1-2> was microinjected into fertilized blastula. Particularly, female and male C57BL/6J mice (Jackson Laboratory, USA) were mated, and 3.5 days after mating, the female mouse was sacrificed by cervical dislocation. Uterus was removed from the sacrificed female mouse and terminal region of the uterus was cut with scissors. Using 1

ml syringe, 1 ml of injection solution containing 20 mM HEPES, 10% FBS, 0.1 mM 2-mercaptoethanol and DMEM was circulated. Blastula was separated from the above uterus using microglasstube under the dissecting microscopy. The separated blastula was transferred into a drop of the injection solution placed on a 35 mm petridish, which was used for the succeeding introduction process. In order to insert the embryonic stem cell clone selected in the above Example <1-3> into the separated blastula, a microinjection was used (Zeiss Co.). That is, 10-15 embryonic stem cell clones were injected into blastocoel of the blastula. The blastula having the clones was mated with a male which was operated on vasectomy, which was implanted in the uterus of 2.5 p.c. surrogate mother mouse in order to induce the generation of a chimera mouse, a kind of heterozygote formed from the embryonic stem cell clones (J1) and the blastula of C57B/6J mouse. For the implantation, the surrogate mother mouse was anesthetized by avertine (1 mg/kg weight), and the abdomen was cut 1 cm; the upper uterus was taken by a pincette and was pulled outside 2 cm; a hole was made on the uterus by a needle, through which the blastula was injected by using a microglasstube; the inner membrane of abdominal cavity was closed with two stitches and the

outside skin was enveloped by a medical clip. The blastula in which the embryonic stem cell clones were inserted was implanted in the uterus of a surrogate mother mouse, followed by incubating for 19 days. So, embryonic stem cell originated cells were fused with blastula originated cells, resulting in the preparation of the chimera mouse having calcium ion channel alpha 1B +/- genotype in its genome.

10 <1-4> Generation of alpha 1B +/- heterozygote mice

Among offspring generated from mating a C57BL/6J female mouse with a male chimera mouse obtained in the above Example <1-3>, genetically stable heterozygote F1 transgenic mice were selected. PCR (polymerase chain reaction) was performed to select heterozygote mice having alpha 1B +/- genotype among them.

DNA for PCR was extracted from mice tails. Particularly, 1.5 cm of each mouse tail was cut and dipped in 0.4 ml of lysis buffer containing 100 mM Tris-HCl (pH 8.0), 5 mM EDTA, 200 mM NaCl and 0.2% SDS. Proteinase K (0.1 mg/ml) was added into the above solution and reacted at 55°C for 5 hours. Thereafter, added 75  $\mu$ l 8 M potassium acetate and 0.4 ml chloroform thereto and shook. The solution was suspended at 4°C for 10 minutes. Supernatant and

sediment were separated by centrifugation at 15,000 rpm. 1 ml of ethanol was added into the 0.4 ml of separated supernatant to precipitate genomic DNA. The precipitated genomic DNA was washed with 70% ethanol. After drying, the genomic DNA was resolved in 50  $\mu$ l of distilled water and used for PCR.

1  $\mu$ l of the above DNA was used as a template for PCR reaction. 10 pmols of Primer 1 (SEQ. ID. No 1), 2 (SEQ. ID. No 2) and 3 (SEQ. ID. No 3) were also used as primers for the PCR. A set of primer 1 and primer 2 was designed to amplify 190 bp DNA fragment of wild-type alpha 1B gene and a set of primer 1 and primer 3 was designed to amplify 320 bp DNA fragment of targeted gene. PCR was performed by 40 cycles as follows: a denaturing step at 94°C for 30 seconds, a primer annealing step at 58°C for 30 seconds and an extension step at 72°C for 30 seconds. After PCR, the PCR product was analyzed by 1.5% agarose gel electrophoresis and ethidium bromide (EtBr) staining. For the control, DNA extracted from wild-type mice was used.

As a result, heterozygote mice having alpha 1B +/- genotype were confirmed by observing that the mice had two different bands; one is a 320 bp size band amplified by targeting alpha 1B gene and the other is a 190 bp size band amplified by wild-type

alpha 1B gene (Lane 3, FIG. 2A).

<1-5> Generation of alpha 1B -/- homozygote mice

Homozygote transgenic mice having alpha 1B -/-  
5 genotype were generated by mating a male and a female  
heterozygote mouse having alpha 1B +/- genotype,  
which were selected in the above Example <1-4>.  
Southern blot and PCR were performed to confirm  
whether the above homozygote transgenic mice had  
10 alpha 1B -/- genotype, and Western blot was also  
performed to confirm that alpha 1B protein was not  
expressed in the transgenic mice.

First, for the Southern blot analysis, genomic  
15 DNA was extracted from transgenic mice tails with the  
same method as used in the above Example <1-4> and  
digested with EcoRI. Hybridization was performed  
using DNA probe obtained from phage DNA of the  
Example <1-1> (□ region, FIG. 1C) and the above  
20 mouse DNA. At this time, genomic DNAs extracted from  
wild-type and transgenic mouse having alpha 1B +/-  
genotype were used as controls.

As shown in FIG. 2B, a 8.0 kb band originated  
from normal alpha 1B gene was detected in wild-type  
25 (+/+) mouse (Lane 1 and 2), and along with the 8.0 kb  
band, a 14.5 kb band was also detected in

heterozygote (+/-) mouse (Lane 3-5). Meanwhile, only  
14.5 kb band originated from an alpha 1B -/- gene was  
detected in homozygote mouse (Lane 6 and 7), from  
which it was confirmed that homozygote transgenic  
5 mouse of the present invention had alpha 1B -/-  
genotype.

Second, after extracting genomic DNA from  
transgenic mice tails with the same method as used in  
10 the above Example <1-4>, PCR was performed, that is;  
1  $\mu$ l of genomic DNA was used as a template and the  
same primers of Example <1-4> were used.

As a result, a 190 bp PCR product amplified  
from normal alpha 1B gene was detected in wild-type  
15 mouse, and along with a 190 bp band, a 320 bp PCR  
product amplified from alpha 1B +/- gene was detected  
in heterozygote mouse. Meanwhile, only 320 bp PCR  
product was detected in homozygote mouse, suggesting  
that the homozygote transgenic mouse of the present  
20 invention had alpha 1B -/- genotype (FIG. 2A).

Third, the present inventors performed Western  
blot analysis in order to confirm that alpha 1B gene  
was not expressed in alpha 1B-knockout mice having  
25 alpha 1B -/- genotype of the present invention.  
Particularly, wild-type and homozygote transgenic

mice were sacrificed by cervical dislocation to isolate cerebrum. The isolated cerebrums were homogenized in cold lysis buffer (50 mM Tris/HCl [pH 7.4], 1 mM EGTA, 1 mM DTT, 1 mM PMSF, complete protease inhibitor cocktail [Boehringer Mannheim], Calpain inhibitors I and II). After low speed centrifugation (1,000 g, 5 minutes), supernatants were centrifuged (28,000 g, 15 minutes) to obtain crude membrane fractions. The crude membrane fractions were separated in gradient SDS PAGE gels (8%-16%), blotted to nitrocellulose membranes (PROTRAN, Schleicher & Schuell), and visualized by the anti-alpha 1B affinity purified polyclonal antibodies (CW21, Vance et al., *J. Biol. Chem.*, 1998, 273:14495-502) by enhanced chemiluminescence.

As shown in FIG. 2C, an alpha 1B protein was not detected in the alpha 1B -/- brain whereas the protein was detected in the wild type (+/+) mouse brain. Therefore, it was confirmed that calcium ion channel alpha 1B protein was not expressed in alpha 1B-knockout mouse of the present invention. The present inventors have deposited the embryo of the transgenic mouse having alpha 1B +/- genotype at Korean Collections for Type Cultures of Korea Research Institute of Bioscience and Biotechnology on Jan. 8, 2002 (Accession No: KCTC 10158BP).

Example 2: Investigation of the effect of N-type  
calcium channel on depression

In order to observe the response of the alpha  
5 1B-knockout mouse (Accession No: KCTC 10158BP)  
deficient in N-type calcium channel through  
depression related animal behavior tests, the present  
inventors performed a forced swimming test and a tail  
suspension test. All the test animals could take  
10 food and water freely in a breeding place where  
temperature and humidity were properly controlled and  
a 12 hour-day/night cycle was given; morning started  
at 8 o'clock. Both female and male of F1 mice were  
used for the tests, and their ages were 8-10 week old.  
15 All the behavior tests were performed by the rules of  
a single blind test. All the tests were performed  
between 9-noon in order to minimize the effect of a  
24-hour cycle. And each mouse was used just once for  
each test.

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<2-1> Reduced immobility in forced swimming test

The forced swimming test was performed  
according to the method of Posolt et al (Posolt, R.D.  
et al., *Eur. J. Pharmacol.*, 1978, 47, 379-391) with a

slight modification. Particularly, water was filled to 15 cm in a 4 l plastic beaker (13 cm × 25 cm), and alpha 1B-knockout mice and control (wild-type) mice were forced to swim therein, causing depression by an inevitable stress. The duration of immobile floating was measured, which reflected the depression-related behavior response. The measurement was made for 15 minutes and data were gathered every 5 minutes. The mean value was used for the analysis.

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As a result, while wild-type mice showed immobile floating position from the 5<sup>th</sup> minute, alpha 1B-knockout mice were swimming all through 15 minutes, showing remarkably short period of immobile floating position, comparing to wild-type mice. From the result, it was confirmed that alpha 1B-knockout mice showed decreased depression response (FIG. 3).

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#### <2-2> Tail suspension test

The tail suspension test was performed according to the method of Steru et al (Steru, L. et al., *Psychopharmacology*, 1985, 85, 367-370). Particularly, the tails of mice were taped and suspended for 7 minutes in the middle of a metal rod, making the heads of mice be located at a height of 25 cm from the bottom of a bucket (35 cm × 40 cm). The

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behavior of each mouse was recorded on videotape and the duration of immobilization time was investigated by a single blind method. A violent behavior was observed for the first 1 minute, which was excluded  
5 for the measurement. And for the next 6 minutes, immobilization time expressing the depression was measured. The mean value was used for the analysis.

As a result, like in the above forced swimming  
10 test, alpha 1B-knockout mice showed remarkably decreased immobilization time, comparing to wild-type mice in the tail suspension test (FIG. 4). From the result, it was confirmed that alpha 1B-knockout mice showed much decreased depression than wild-type mice.

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#### INDUSTRIAL APPLICABILITY

As explained hereinbefore, a transgenic mouse deficient in N-type calcium channel showed much decreased depression than a wild-type mouse,  
20 suggesting that depression could be decreased by inhibiting the activity of N-type calcium channel gene. Therefore, substances inhibiting the activity of N-type calcium channel gene or inhibiting the function of N-type calcium channel, a product of the  
25 above gene, can be effectively used as a treatment

agent for depression.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as  
5 a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not  
10 depart from the spirit and scope of the invention as set forth in the appended claims.